



Serological markers of autoimmunity in women with polycystic ovary syndrome

Wasan Wajdi Ibrahim¹, Ekhlas Jabbar Kadhim¹, Nada Saeed Abbas², Saba Ryadh Younis¹, Hayder Adnan Fawzi*²

¹Department of Obstetrics and Gynecology, College of Medicine, Baghdad University, Iraq

²Department of Clinical Pharmacy, Medical hospital, Ministry of Health, Baghdad-Iraq



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ABSTRACT

In the present study, we evaluate the serum level of common autoimmunologic markers in women with polycystic ovary syndrome (PCOS) and study their relationship with hormonal parameters. The study was an observational case-control study, done in a Tertiary referral hospital, the study included 50 women with polycystic ovary syndrome and 50 matched control. Serum levels of Antinuclear Antibody (ANA) and anti-double-stranded DNA (dsDNA) were significantly higher in polycystic ovary syndrome women compared to control. Also, luteinizing hormone (LH), follicle stimulating hormone (FSH), and LH/FSH ratio were significantly higher in polycystic ovary syndrome women compared to control. dsDNA had excellent ability to differentiate PCOS from control (AUC=0.901) while ANA had good discrimination ability (AUC=0.809). There was a significant direct relationship between ANA, dsDNA, and TSH with FSH in PCOS women, also a significant direct relationship between ANA and TSH with LH, while DsDNA did not correlate with LH. In conclusion, there is a clear relationship between immunological markers (ANA, dsDNA) with polycystic ovary syndrome in various components of the disease, dsDNA offer better ability than ANA as a predictor of PCOS, indicating that dsDNA can be used as a non-invasive diagnostic tool for PCOS.

*Corresponding Author

Name: Hayder Adnan Fawzi

Phone:

Email: Hayder.adnan2010@gmail.com

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) affects 5 to 10% of women of childbearing age and is the most common cause of anovulatory infertility in developed countries; it is characterized by the presence of two or three of the following features: Oligo-

ovulation or anovulation, hyperandrogenism, and polycystic ovaries. (NESTLER *et al.*, 1998).

The first evidence for antinuclear antibodies arose in 1948 when Hargraves, Richmond, and Morton discovered the LE cell (HARGRAVES *et al.*, 1948). These abnormal cells, which are found in the bone marrow of persons with systemic lupus erythematosus (SLE), are categorized as polymorph nuclear leukocytes with phagocytized whole nuclei (SHAO and COHEN, 2011). Subsequently, in 1957, antibodies to dsDNA were the first autoantibodies to be identified in patients with SLE (STOLLAR, 1989). Antinuclear antibodies are a group of antibodies found against a variety of nuclear antigens have been detected in the serum of patients with autoimmune diseases. An inflammatory response will increase the production of ANA which can be measured and used as a marker for disease activity in sera (DEHAGHANI *et al.*, 2013).

In autoimmunity, there is a failure in mechanisms responsible for self-tolerance and regulation with overstimulation of immune response against self-components. Also, its characterized by induction of autoreactive cells toward antigens (e.g., antibodies). Autoimmunity had been classified as organ-specific and non-organ specific. The inflammatory process, hyperstimulation of immunity, and the process of tissue destruction expose intracellular antigens leads to the production of ANA, which is a hallmark of autoimmune disorders. This marker (i.e., ANA) has been detected in several autoimmune disorders like systemic lupus erythematosus, Sjogren's syndrome, autoimmune hepatitis, dermatomyositis, and polymyositis. Low levels of progesterone in PCOS lead to overstimulates the immune system and causing uncontrolled production of autoantibodies, and it will lead to an autoimmune disorder (MOBEEN *et al.*, 2016). The current work aimed to evaluate autoimmune markers ANA and dsDNA in women with PCOS and to evaluate the correlation between these autoantibodies with serum FSH, LH, LH/FSH ratio and serum TSH levels in polycystic ovary syndrome.

MATERIALS AND METHODS

Patient selection and study design

This case-control study conducted in Baghdad teaching hospital/ Medical city complex for six months starting from 1st January 2017 till 30th June 2017. The study included 100 healthy women which were divided into 2 groups: group A included 50 women diagnosed with PCOS according to the 2003 Rotterdam Criteria (R.O.T.T.E.R.D.A.M., 2004) and were recruited from the infertility clinic unite in Baghdad teaching hospital and group B included 50 fertile control women seeking contraception in the outpatient clinic without having PCOS were (50 women with PCOS and 50 normal age-matched control), the women's age between 19 to 33 years.

Extensive general, abdominal, and pelvic examinations were performed for all included women. Transvaginal ultrasound was performed to evaluate the ovaries and uterus.

The study was approved by the scientific council of Gynecology and obstetrics Arabic board, written inform consent obtained from the patients, and this study done in accordance of Helsinki Declaration.

Inclusion criteria

1. Age between 18 to 40 years
2. Confirmed

Exclusion criteria

1. A history of medical treatment, hyperthyroidism, hyperprolactinemia, or chronic hypertension
2. Any hormonal treatment during the previous three months before the study or any medication affecting ANA and dsDNA levels, such as antipsychotics (e.g., chlorpromazine, haloperidol, and clozapine)
3. Drug-induced lupus associated with pyrazinamide or sulfadiazine.
4. Aromatase inhibitors (e.g., letrozole and anastrozole), which increase the incidence of autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Sample collection

Blood samples were obtained in the morning to determine serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and serological tests. Serum ANA levels were measured by immunometric enzyme immunoassay against nucleosomes, and ELISA measured IgG class autoantibodies against dsDNA.

Serum level of ANA was considered to be positive if above or equal to 10 IU/mL for autoimmune disease, while for dsDNA levels below 30 IU/mL considered to be negative, levels between 30 – 75 IU/mL considered to be borderline and levels above 75 IU/mL is positive for autoimmune disease.

Statistical analysis

Discrete variables presented using their numbers and percentages; Chi-square test was used to analyze the discrete variables (or Fisher exact test when Chi-square test is not valid; due to low sample size < 20 and if two or more with an expected frequency is less than 5). Two samples t-test was used to analyze the differences in means between two groups (if both follow a normal distribution with no significant outlier). Receiver operator curve used to see the validity of different parameters in separating cases with torsion from none torsion and area under the curve, i.e. AUC and its p-value prescribe this validity (if AUC \geq 0.9 mean excellent test, 0.8 – 0.89 means good test, 0.7 – 0.79 fair test otherwise unacceptable). Linear regression analysis was performed to assess the relationship between different variables. SPSS 22 (Chicago, IL), MedClac 14.8.1, GraphPad Prism 7.0 software package was used to

make the statistical analysis, p-value considered to be significant if less than 0.05.

Results

Serum levels of LH, LH/FSH ratio, TSH, ANA, dsDNA was significantly higher in PCOS compared to control, while FSH was significantly lower in PCOS compared to control, as illustrated in Table 1 .

Table 1: Assessment of demographic and immunological markers in PCOS and control

Variables	PCOS	Control	P value
Number	50	50	-
Maternal age (years), mean ± SD	25.3 ± 2.4	26.2 ± 3.5	0.147
BMI (kg/m2), mean ± SD	26.3 ± 2.3	26.1 ± 1.9	0.637
FSH, mean ± SD	6.1 ± 2.3	7.4 ± 2.3	0.005 [S]
LH, mean ± SD	10.5 ± 2.6	4.9 ± 1.2	<0.001 [S]
LH/FSH ratio, mean ± SD	1.9 ± 0.8	0.7 ± 0.2	<0.001 [S]
TSH, mean ± SD	1.6 ± 0.8	1.1 ± 0.5	<0.001 [S]
ANA (IU/ml); mean ± SD	8.0 ± 2.7	5.1 ± 2.6	<0.001 [S]
DsDNA (IU/ml) ; mean ± SD	54.2 ± 20.3	24.0 ± 15.0	<0.001 [S]

Anti dsDNA had higher validity than ANA for differentiating PCOS (since it has higher AUC), both had similar sensitivity, but dsDNA had higher specificity than ANA. Since PPV and NPV for dsDNA is 84% indicating it has the similar ability for confirmation and exclusion of PCOS, as illustrated in Table 2 and Figure 1.

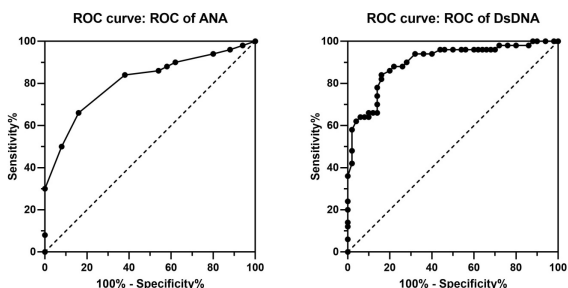


Figure 1: ROC for immunological parameters for discrimination of PCOS from control

There was a significant direct relationship between ANA, dsDNA, and TSH with FSH in PCOS women, also a significant direct relationship between ANA and

TSH with LH, while DsDNA did not correlate with LH, as illustrated in Table 3.

Table 3: relationship between hormones and immunological markers in PCOS women

Variables	FSH		LH	
	r	P value	r	P value
ANA	0.341	0.016 [S]	0.351	0.012 [S]
DsDNA	0.331	0.019 [S]	0.252	0.077
TSH	0.484	<0.001 [S]	0.359	0.011 [S]

r: correlation coefficient

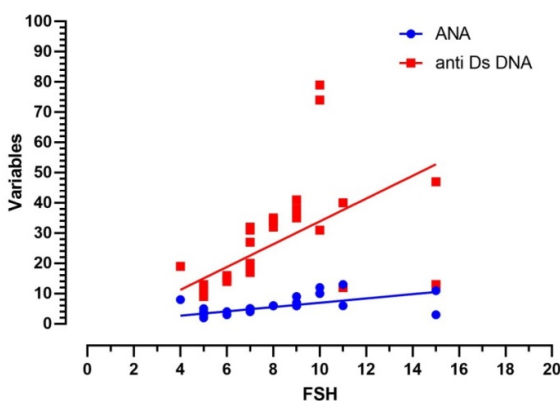


Figure 2: scatterplot of the relationship between FSH vs.ANA and dsDNA (ANA: r = 0.398, p-value =0.004, dsDNA: r = 0.667, p-value <0.001)

RESULTS AND DISCUSSION

In the current study, two of the most common immunological markers were examined (antinuclear antibodies and double-stranded DNA) both represent the basic markers used in the diagnosis of autoimmune diseases. In this study serum ANA was significantly higher in PCOS group compared to control (8.0 ± 2.7 vs. 5.1 ± 2.6 IU/ml respectively), these findings were in agreement with Reimand et al. (REIMAND *et al.*, 2001) in their case-control study which includes 108 female cases with various reproductive diseases from this group 20 women had PCOS 6 women had elevated ANA (6/20, 30%) compared to 14 out of 392 (3.6%) of normal age matched control women and this was statistically significant, additionally the current study agreed with a recent study in Egypt published in 2015 in which Makled et al. (MAKLED *et al.*, 2015) performed a case control study on 50 PCOS and 50 age-matched control women and found that PCOS women had significantly higher ANA titer compared

Table 2: Validity of immunological markers for differentiation PCOS from control

Variables	AUC	Cut off	P value	Sensitivity	Specificity	PPV	NPV
ANA	0.809	≥5	<0.001	84%	66%	71.2	80.5
DsDNA	0.901	≥36	<0.001	84%	84%	84%	84%

to control (9.0 ± 6.1 vs. 5.4 ± 2.3 U/ml) with 36.0% of PCOS had elevated ANA vs. 6.0% in the control group, on the other hand, the results of this study concerning ANA was in disagreement with Hefler-Frischmuth et al. (Hefler-Frischmuth et al., 2010), they performed case control study with 109 PCOS against 109 matched control in Austria 2010; which they reported no differences in ANA levels between the groups this is the single study that reported no difference in ANA between PCOS compared to control which can be attributed to differences in selection criteria of the patients.

The result of this study showed that DsDNA was significantly higher in PCOS compared to control (54.2 ± 20.3 vs. 24.0 ± 15.0 IU/ml) and that 28.0% of PCOS had elevated DsDNA compared to 2.0% in control, which is agreement with Makled et al (MAKLED et al., 2015) (56.3 ± 25.7 vs. 26.0 ± 10.8 IU/ml for PCOS and control respectively) and in agreement with Hefler-Frischmuth et al. (HEFLER-FRISCHMUTH et al., 2010) with both studies showing that DsDNA is significantly higher in PCOS compared to matched normal control, which in agreement with the result of the current study in which DsDNA had higher prediction of PCOS compared to ANA (AUC in ROC test was higher in DSDNA vs. ANA 0.901 vs. 0.809) indicating its consistency in elevation in PCOS and ANA had more variable elevation (MAKLED et al., 2015) (HEFLER-FRISCHMUTH et al., 2010).

In this study serum ANA significantly correlated with FSH ($r=0.341$, $p=0.016$), with LH ($r=0.351$, $p=0.012$), and with TSH ($r=0.398$, $p=0.004$); Makled et al. (MAKLED et al., 2015) agree with our findings only in cases TSH correlation with ANA which they found to be significant, while ANA correlation with FSH and LH appear to be not significant, Hefler-Frischmuth et al. (HEFLER-FRISCHMUTH et al., 2010) also found a significant correlation between TSH and ANA ($p=0.03$), in their study only TSH correlation with ANA which is more commonly elevated in immunological conditions while LH and FSH had lower immunogenicity compared to TSH.

In this study serum DsDNA also significantly correlated with FSH ($r=0.331$, $p=0.019$) with TSH ($r=0.667$, $p<0.001$) and not significantly with LH ($r=0.252$, $p=0.077$), Makled et al (MAKLED et al.,

2015) disagree with our findings concerning the correlation between DsDNA with endocrine markers in which they found no such correlation to be found in (FSH, LH and TSH) while Hefler-Frischmuth et al (HEFLER-FRISCHMUTH et al., 2010) agree with our findings except they also reported a significant correlation between DsDNA with LH in addition to significant correlation with FSH and TSH, since DsDNA is highly correlated with PCOS so it will be expected to highly correlate with the hormonal component of PCOS (TSH and FSH), other than that no clear mechanism can explain such observation.

The findings of this study in conjunction with previously reported studies (REIMAND et al., 2001) (MAKLED et al., 2015) (HEFLER-FRISCHMUTH et al., 2010) indicate a significant link between autoimmunity and PCOS, Glintborg et al. (GLINTBORG and ANDERSEN, 2010) reported an increased inflammatory response with overexpression in the immune mediator in PCOS patients, Fulghesu et al (FULGHESU et al., 2011) show an association between monocytes in patients with insulin - resistance PCOS, and with interleukin 6 (which is inflammatory cytokine), other postulated the low levels of progesterone as a reason for immune overstimulation (PETRIKOVA et al., 2010) so these findings can indicate the elevation in ANA and DsDNA levels possibly caused by immune overstimulation and inflammation, Dehaghani et al. (DEHAGHANI et al., 2013) in their study of ovarian electrocauterization (to destroy parts of ovary that affected by PCOS) their observation of increase ANA levels after the procedure imply that manipulation in ovary tissues in women with PCOS provoke inflammatory response or increase release of inflammatory mediators (that either directly elevate ANA or DsDNA or precipitate their elevation) leading to autoimmunity, since one of the accepted theory of autoimmunity is tissue destruction (MACKAY et al., 2008). No clear antibodies were found in previous studies directed to ovarian tissue but a status of an overall increased immune activation without specific antibody, which can be caused by different mechanisms that need to be clarified (REIMAND et al., 2001).

In this study we evaluated ANA and DsDNA as predictor of PCOS with found if ANA ≥ 5 had 84% sensitivity and 66% specificity indicating that ANA had

higher sensitivity compared to its specificity and its better as a screening tool, while DsDNA with cut point ≥ 36 having 84% sensitivity and specificity indicating it had similar clinical utility as confirmatory and screening diagnostic tool, Malked et al. (MAKLED *et al.*, 2015) in their study showed similar findings concerning the use of both DsDNA and ANA as diagnostic tool for PCOS; however they found DsDNA had an optimal cut point of >74 and for ANA >9.8 .

CONCLUSION

There is a clear relationship between immunological markers (ANA, DsDNA) with polycystic ovary syndrome in various components of the disease, dsDNA offer better ability than ANA as a predictor of PCOS, indicating that dsDNA can be used as non-invasive diagnostic tool for PCOS.

CONFLICTS OF INTERESTS

All authors have none to declare.

Author contributions

All author contributed equally.

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